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Disorders of puberty: Inactivating and activating molecular mutations

Linda Anne DiMeglio, MD, and Ora Hirsch Pescovitz, MD

Recent developments have increased our understanding of the molecular mechanisms that are responsible for several disorders of puberty. Specific gene mutations have been identified in three syndromes, one that is associated with delayed puberty (Kallmann syndrome) and two that are associated with precocious puberty (McCune-Albright syndrome and familial male precocious puberty). Mutations in the *KAL* gene have been shown to be responsible for cases of X-linked Kallmann syndrome. This gene encodes a protein that is believed to be involved in neural target recognition and protease inhibition. In McCune-Albright syndrome, heterozygous, postzygotic somatic mutations of the α-subunit of the stimulatory guanine nucleotide binding protein G_s have been shown to stimulate constitutive G protein activation and long-term cyclic adenosine monophosphate production. Similarly, familial male precocious puberty has been linked to gain-in-function mutations that result in increased levels of cyclic adenosine monophosphate; however, these mutations are found in the luteinizing hormone receptor gene itself. The clinical manifestations and the recent molecular advances in each of these three syndromes are explored. (J Pediatr 1997;131:S8-12)

Recent advances have increased our understanding of the molecular mechanisms of several disorders of puberty. Gene mutations have been described in three separate syndromes: Kallmann syndrome, McCune-Albright syndrome, and familial male precocious puberty. In this article, we review the clinical manifestations and recent molecular discoveries in each of these three syndromes.

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KALLMANN SYNDROME

Kallmann syndrome was first described in 1856 by the Spanish anatomist Maestre de San Juan, who reported a person with small testes and anosmia (reviewed in reference 1). In 1944 Kallmann, an American geneticist, noted coinheritance of hypogonadism and anosmia in three families. In 1954 the Swiss anatomist deMorsier described absent olfactory bulbs and tracts in several male patients with hypogonadism. In French-speaking countries the syndrome is known as *syndrome de deMorsier*.

Kallmann syndrome is the most common form of isolated gonadotropin deficiency, occurring in 1 in 10,000 males and 1 in 50,000 females.[2] Affected persons have hypogonadism and either anosmia or hyposmia. Other associated features include neurologic abnormalities (uncoordinated eye movements, cerebellar ataxia, sensorineural deafness, synkinesis, spatial attention abnormalities, mental retardation) and somatic defects (pes cavus, cleft lip and palate, unilateral renal agenesis).

cAMP	Cyclic adenosine monophosphate
GnRH	Gonadotropin-releasing hormone
LH	Luteinizing hormone

Sporadic as well as autosomal-dominant, autosomal-recessive, and X-linked recessive modes of inheritance have been described. The clinical expression of the disease varies in persons with inherited forms, and differential expression has even been reported in monozygotic twins.

The anosmia in Kallmann syndrome appears usually to result from aplasia or hypoplasia of the olfactory bulbs and tracts. However, some persons with the syndrome have normal olfactory anatomy. The hypogonadism in Kallmann syndrome results from variable reductions in hypothalamic secretion of gonadotropin-releasing hormone (GnRH). The connection between anosmia and hypogonadism in Kallmann syndrome can be explained by an embryologic link. GnRH-synthesizing neurons arise within the nasal epithelium. From there they appear to migrate to the olfactory placode and then travel along the olfactory epithelium–forebrain axis to the preoptic and hypothalamic areas.

The gene responsible for many of the X-linked cases of Kallmann syndrome was identified independently by two groups in 1991.[3, 4] Studies in men with X-linked Kallmann syndrome have subsequently shown many heterogeneous mutations of

this region. Located at Xp22.3, adjacent to genes for X-linked ichthyosis and X-linked chondrodysplasia punctata, the *KAL* gene encodes a 680–amino acid extracellular matrix protein.

The KAL protein has features both of serine protease inhibitors that modulate axonal outgrowth, pathfinding, and target recognition and of cell adhesion molecules that are involved in axonal extension. It appears to be involved in GnRH–secreting neuron migration and in olfactory axon targeting (reviewed in reference 2). This is substantiated by pathologic evidence. In persons with Kallmann syndrome, the olfactory epithelium is thinner and contains fewer neurons than in normal adults. Those that are present have no cilia, appear immature, and show signs of degeneration. A male fetus with a large Xp22.3 deletion has been studied at 19 weeks of gestation; his GnRH and olfactory axons had left the olfactory epithelium but had formed only tangles on the dorsal side of the cribriform plate.[5] He had no GnRH neurons, nor any axon terminals of his olfactory, terminalis, or vomeronasal nerves.

The molecular aspects of Kallmann syndrome are difficult to study for many reasons. The *KAL* gene is conserved between human beings and a number of other species, but there is no murine model because a similar gene is not present in mice or rats. There is also a significant dissociation between the genotype and the phenotype in persons with the syndrome. Even among those who are believed to have X-linked Kallmann syndrome, no *KAL* gene abnormality has yet been identified in 50%. It has been proposed that these persons have an abnormal autosomal gene with sex-limited expression or that perhaps there is another X-linked gene. We now understand some of the molecular mechanisms of Kallmann syndrome, but much about it remains a mystery.

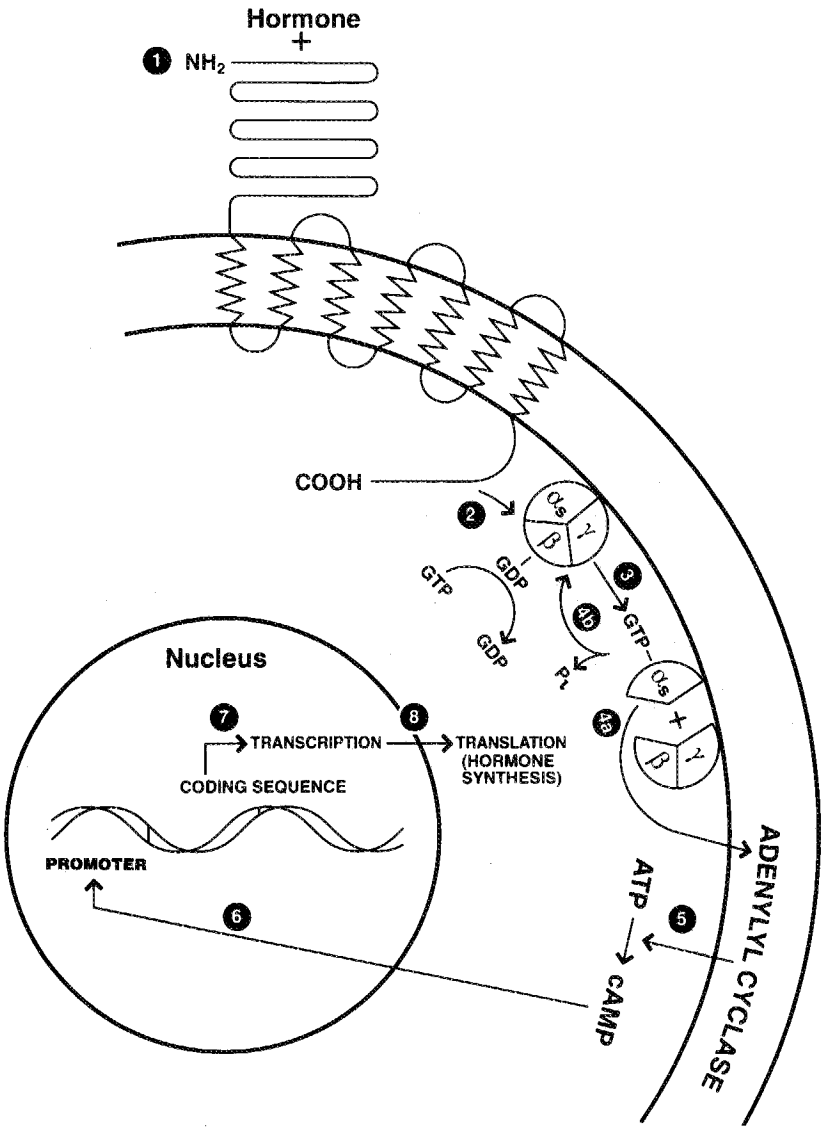

Fig. 1. Mechanism of gonadotropin-induced synthesis of sex steroids in Leydig or granulosa cells. Gonadotropins (LH, follicle-stimulating hormone) bind to and activate their specific receptors *(1)*, which in turn induce guanosine triphosphate *(GTP)* to displace guanosine diphosphate *(GDP)* from the heterotrimeric membrane-bound inactive G protein *(2)*. The activated G protein then dissociates into a GTP-bound $G_s\alpha$ subunit *(3)*, which stimulates adenylyl cyclase *(4a)* before its intrinsic guanosine triphosphatase activity hydrolyzes GTP to form the GDP-bound inactive heterotrimeric G protein *(4b)*. Activation of adenylyl cyclase results in the generation of cAMP from adenosine triphosphate *(ATP) (5)*. The cAMP then directly or indirectly causes gene activation *(6)*, transcription *(7)*, and translation (i.e., synthesis of testosterone or estradiol *[8]*). (From Shankar RR, Pescovitz OH. Adv Endocrinol Metab 1995;6:55-89.)

McCUNE-ALBRIGHT SYNDROME

In 1937, McCune and Bruch[6] described a 4-year-old girl with bowed legs, irregular macules, uterine bleeding, breast enlargement, and axillary and pubic hair. Albright et al.[7] then described a distinct syndrome characterized by a clinical triad: bone lesions with features of osteitis fibrosa on histologic examination; brown nonelevated pigmented skin lesions, often on the same side as the bone lesions; and endocrine dysfunction, characterized by precocious puberty in girls. The syndrome has since been called McCune-Albright syndrome.

McCune-Albright syndrome usually occurs sporadically and is more common in girls than in boys.[8] The precocious puberty can be preceded by pigmented skin lesions and polyostotic fibrous bone dysplasia. The skin lesions, found in about 90% of children with McCune-Albright

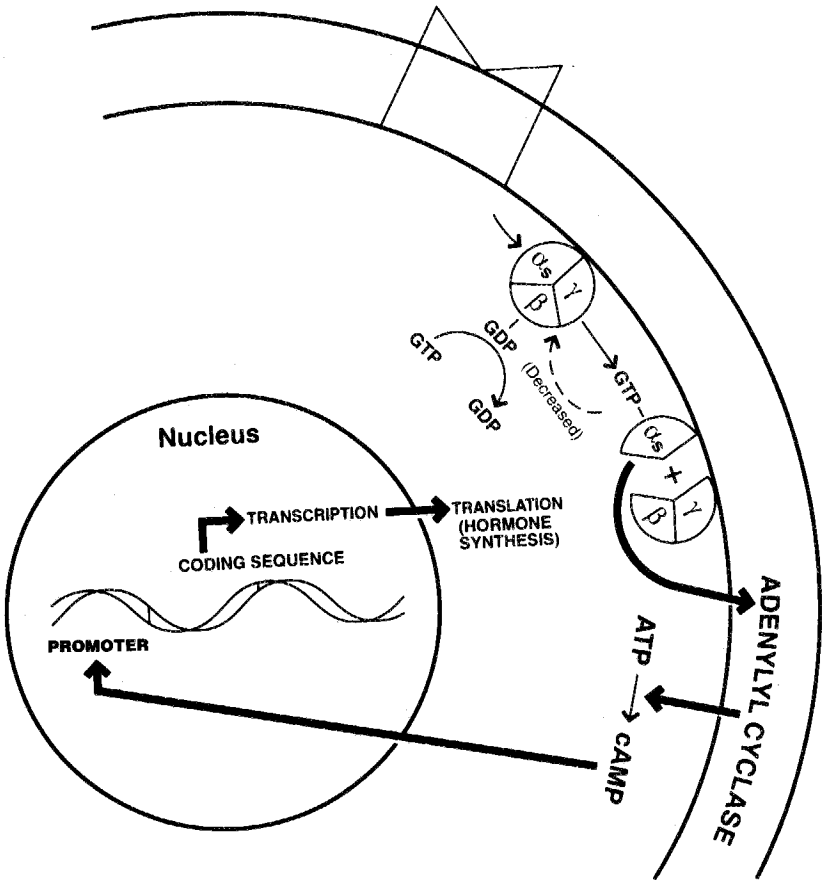

Fig. 2. Mechanism of gonadotropin-independent synthesis of sex steroids in McCune-Albright syndrome. In McCune-Albright syndrome the $G_s\alpha$ subunit of the G protein is mutated at position 201 (histidine or cysteine substitution for arginine), which results in a reduction in intrinsic guanosine triphosphatase activity. This causes unregulated activation of adenylyl cyclase and constitutive cAMP production, resulting in precocious steroid production. See Fig. 1 for abbreviations. (From Shankar RR, Pescovitz OH. Adv Endocrinol Metab 1995;6:55-89.)

syndrome, are irregularly shaped café-au-lait macules that can occur anywhere. They rarely cross the midline and may correspond to the side of the bony lesions. The severity of the fibrous dysplasia can range from subtle changes that can be discerned only by bone scan to fractures that produce skeletal deformity and significant disability. Vaginal bleeding is often the first manifestation of precocious puberty in girls with McCune-Albright syndrome. Unlike in children with central precocious puberty, which is usually progressive, the secondary sexual characteristics in children with McCune-Albright syndrome typically wax and wane.

McCune-Albright syndrome is associated with extragonadal endocrinopathies. Hyperthyroidism occurs in 20% to 40% of patients. There have also been more than two dozen reports of excessive secretion of growth hormone in McCune-Albright syndrome; most of these cases are associated with hyperprolactinemia. Cushing syndrome associated with nodular adrenal hyperplasia has also been reported in several patients. Other nonendocrine manifestations of McCune-Albright syndrome include hypophosphatemia; hepatobiliary dysfunction; thymic, splenic, and pancreatic hyperplasia; acute pancreatitis; gastrointestinal polyps; abnormal cardiac muscle cells; and sudden or premature death.[8, 9]

The clinical manifestations of McCune-Albright syndrome appear to result from autonomous hyperactivity of tissues that produce products regulated by intracellular accumulation of cyclic adenosine monophosphate (cAMP). This constitutive overproduction of cAMP is mediated by activating mutations of the stimulatory guanine nucleotide binding protein (G_s), which is a heterotrimeric protein made of α, β, and γ subunits. The activated α-subunit stimulates adenylate cyclase, increasing the production of cAMP. It also acts as a guanosine triphosphatase by catalyzing the hydrolysis of bound guanosine triphosphate to guanosine diphosphate (Fig. 1). This hydrolytic process results in autoinactivation and a return of the cell to its baseline state. Mutations in positions 201 and 227 of the $G_s\alpha$ gene reduce this intrinsic guanosine triphosphatase activity, thereby stimulating constitutive activation of the G_s protein and increased intracellular accumulation of cAMP (Fig. 2). Increased cAMP is known to produce endocrine hyperplasia and hyperfunction. Mutations of $G_s\alpha$, sometimes referred to as *gsp* mutations, may convert the gene into a *gsp* oncogene and have been associated with autonomous hormone-producing tumors.[10]

Weinstein et al.[11] originally studied McCune-Albright syndrome in four patients, using amplification of genomic DNA by polymerase chain reaction, denaturing of gradient gel electrophoresis, and allele-specific oligonucleotide hybridization to show mutations in position 201 of exon 8 of one allele of the $G_s\alpha$ gene. In two patients, histidine was substituted for arginine; in the other two patients, cysteine replaced arginine. The authors found the same mutations in numerous other endocrine and nonendocrine tissues. In 1994, Shenker et al.[12] were the first to recover this mutation from the dysplastic bone lesions of patients with McCune-Albright syndrome.

Subsequently, Candeliere et al.[13] also found increased expression of the c-*fos* proto-oncogene in these lesions. Bone is a known target tissue for the c-*fos* proto-oncogene, which forms heterodimers with other proteins such as c-*jun*, to form the transcription factor AP-1. AP-1 then

binds to target gene promoter regions and modulates their expression. Other specific molecular consequences of increased cAMP in persons with McCune-Albright syndrome have not yet been characterized.

It is also possible that not all the changes of McCune-Albright syndrome are due solely to increased cAMP.[9] The G_s protein is known to have several functions, and a mutation in its α subunit may alter other intracellular processes. If it impairs the ability of the G_s protein to modulate ion channels and a calcium pump or affects its role in cellular differentiation and intracellular membrane trafficking, it might produce other global effects. Mutations at position 201 have already been shown to have the following effects on $G_s\alpha$: decreasing its membrane attachment, increasing its degradation, and altering its interaction with the other G_s protein subunits.

The abundance of mutant alleles varies between regions of affected tissues.[11, 12] Histologically abnormal tissues contain a higher proportion of $G_s\alpha$ mutations than does normal-appearing tissue, producing a mosaic pattern. This affirms the hypothesis, first proposed by Happle,[14] that McCune-Albright syndrome results from a postzygotic somatic mutation. If the $G_s\alpha$ mutation occurs early in development, its manifestations are more global; if it occurs later, the symptoms are more focal. Unique distributions of mutant cells within individuals explain the various clinical constellations of symptoms.

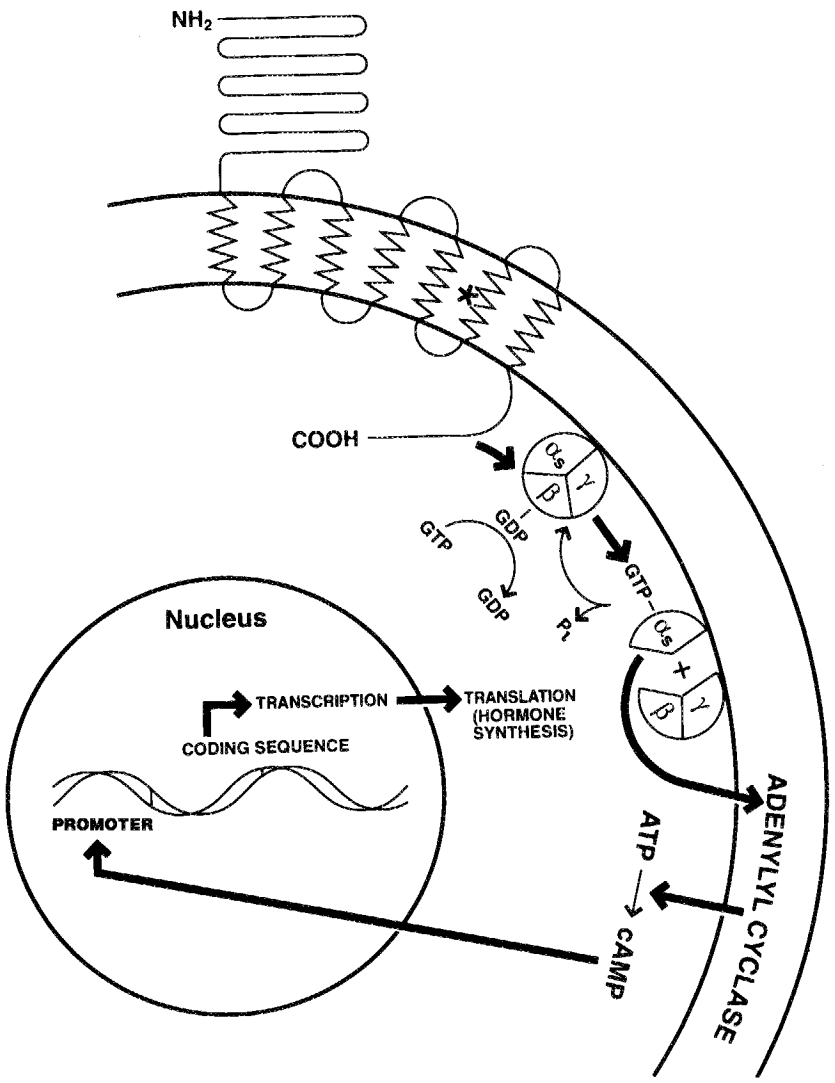

Fig. 3. Mechanism of gonadotropin-independent synthesis of testosterone in familial male precocious puberty. Substitution of glycine for aspartate at position 578 in the sixth transmembrane helix of the luteinizing hormone receptor causes the receptor to be turned on constitutively and results in long-term activation of the G proteins, cAMP, and testosterone production. See Fig. 1 for abbreviations. (From Shankar RR, Pescovitz OH. Adv Endocrinol Metab 1995;6:55-89.)

FAMILIAL MALE PRECOCIOUS PUBERTY

Familial male precocious puberty was first described by Stone[15] in 1852. It can be inherited in an autosomal-dominant sex-limited fashion, but it can also occur sporadically. Carrier women can pass the disease on to their sons but do not themselves manifest any of its features. Affected boys have precocious sexual development that includes modest testicular enlargement with active spermatogenesis, axillary and pubic hair development, penile growth, muscular development, acne, libido, and a growth spurt. The onset is generally in the first to fourth years of life and is typically associated with advanced skeletal maturation. The only long-term consequence of familial male precocious puberty appears to be compromised final adult height because of premature epiphyseal fusion. In 1983, Rosenthal et al.[16] coined the term testotoxicosis to refer to this condition.

Children with familial male precocious puberty have pubertal serum testosterone levels, despite low, nonpulsatile levels of luteinizing hormone. Egli et al.[17] performed GnRH stimulation tests on two men with familial male precocious puberty and found that, unlike in boys with familial male precocious puberty who have suppressed gonadotropins, the responses to GnRH in the men were normal. This suggests that in adults a normal hypothalamic-pituitary-testicular axis overrides the primary testicular defect.

Shenker et al.[18] and Kremer et al.[19] used the polymerase chain reaction to am-

plify genomic DNA from subjects with familial male precocious puberty and their unaffected family members. The authors examined the DNA that encodes amino acid residues 441 to 594 of the LH receptor, a region that encompasses most of the third through sixth transmembrane helices. Using temperature gradient gel electrophoresis, they detected heterozygous mutations. Sequencing showed a substitution of glycine for aspartate at position 578 of the sixth transmembrane helix in affected members from three families.

To show that this receptor mutation could cause the hypersecretion of testosterone seen in males with familial male precocious puberty, Shenker et al.[18] transiently transfected COS cells with the mutant LH receptor. Even in the absence of hormonal stimulus, these cells produced markedly increased levels of cAMP, which suggests that autonomous Leydig cell activity results from constitutive activation of the mutant LH receptor (Fig. 3). When these transiently transfected cells were exposed to human chorionic gonadotropin, cAMP production rose in a manner similar to that in cells transfected with the normal LH receptor. In ligand-binding studies, cells transfected with the Asp → Gly mutation have higher basal cAMP levels.[20] Other secondary messenger systems that involve activation have also been implicated in this process of abnormal steroidogenesis.

To date, 11 different missense point mutations in the LH receptor that also produce the familial male precocious puberty phenotype have been identified.[21] It is believed that the familial male precocious puberty phenotype does not develop in girls with a heterozygous activating LH receptor mutation because both LH action and follicle-stimulating hormone action are required for ovarian steroidogenesis.[22]

Conclusion

It is likely that the advances in our comprehension of the mechanisms that underlie these three disorders of puberty will aid in our understanding of a wide range of other diseases. Activating mutations of G protein–coupled receptors have already been discovered in hyperfunctioning thyroid adenomas and in inherited forms of hypercalcemia and hypocalcemia. These discoveries can now be expected to lead to the development of targeted forms of therapy for these disorders of puberty.

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